

**BOX PATENT
APPLICATION**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: C.L. Steele et al. Attorney Docket No.: WSUR118414
Title: MONOTERPENE SYNTHASES FROM GRAND FIR (ABIES GRANDIS)

PRELIMINARY AMENDMENT

Seattle, Washington 98101

TO THE COMMISSIONER FOR PATENTS:

In the Specification:

Please rewrite and divide the section beginning on page 1, line 2, into two sections with separate headings as follows:

CROSS-REFERENCES TO RELATED APPLICATIONS

The present application is a divisional of U.S. Application No. 09/360,545, filed on July 26, 1999, which is a continuation-in-part of International Application No. US98/14528, filed on July 10, 1998, which was published in English on January 21, 1999, and which claims benefit of priority from United States Provisional Application No. 60/052,249, filed on July 11, 1997.

**STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT**

This invention was funded in part by grant GM-3135A from the National Institutes of Health and by grant 97-35302-4432 from the United States Department of Agriculture. The government has certain rights in this invention.

In the Claims:

Amend Claim 67 as shown below and cancel Claims 34-38, 74, and 81. Pending claims are as follows:

1. An isolated nucleic acid molecule that hybridizes under stringent conditions to a probe selected from the group consisting of the portion of SEQ ID NO:3 extending from nucleotide 1560 to nucleotide 1694 and the portion of SEQ ID NO:5 extending from nucleotide 1180 to nucleotide 1302.

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1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

2. An isolated nucleic acid molecule of Claim 1 that hybridizes under stringent conditions to the portion of SEQ ID NO:3 extending from nucleotide 1560 to nucleotide 1694.

3. An isolated nucleic acid molecule of Claim 1 that hybridizes under stringent conditions to the portion of SEQ ID NO:5 extending from nucleotide 1180 to nucleotide 1302.

4. An isolated nucleic acid molecule of Claim 1 encoding a (-)-camphene synthase.

5. An isolated nucleic acid molecule of Claim 1 encoding a gymnosperm (-)-camphene synthase.

6. An isolated nucleic acid molecule of Claim 1 encoding a Grand fir (-)-camphene synthase.

7. An isolated nucleic acid molecule of Claim 1 which encodes the amino acid sequence of SEQ ID NO:65.

8. An isolated nucleic acid molecule of Claim 1 having the sequence of SEQ ID NO:64.

9. An isolated nucleic acid molecule of Claim 1 encoding a (-)- β -phellandrene synthase.

10. An isolated nucleic acid molecule of Claim 1 encoding a gymnosperm (-)- β -phellandrene synthase.

11. An isolated nucleic acid molecule of Claim 1 encoding a Grand fir (-)- β -phellandrene synthase.

12. An isolated nucleic acid molecule of Claim 1 which encodes the amino acid sequence of SEQ ID NO:67.

13. An isolated nucleic acid molecule of Claim 1 having the sequence of SEQ ID NO:66.

14. An isolated nucleic acid molecule of Claim 1 encoding a terpinolene synthase.
15. An isolated nucleic acid molecule of Claim 1 encoding a gymnosperm terpinolene synthase.
16. An isolated nucleic acid molecule of Claim 1 encoding a Grand fir terpinolene synthase.
17. An isolated nucleic acid molecule of Claim 1 which encodes the amino acid sequence of SEQ ID NO:78.
18. An isolated nucleic acid molecule of Claim 1 having the sequence of SEQ ID NO:77.
19. An isolated nucleic acid molecule of Claim 1 encoding a (-)-limonene/(-)- α -pinene synthase.
20. An isolated nucleic acid molecule of Claim 1 encoding a gymnosperm (-)-limonene/(-)- α -pinene synthase.
21. An isolated nucleic acid molecule of Claim 1 encoding a Grand fir (-)-limonene/(-)- α -pinene synthase.
22. An isolated nucleic acid molecule of Claim 1 which encodes the amino acid sequence of SEQ ID NO:69.
23. An isolated nucleic acid molecule of Claim 1 having the sequence of SEQ ID NO:68.
24. An isolated nucleic acid molecule of Claim 1 encoding a myrcene synthase.
25. An isolated nucleic acid molecule of Claim 1 encoding a gymnosperm myrcene synthase.
26. An isolated nucleic acid molecule of Claim 1 encoding a Grand fir myrcene synthase.
27. An isolated nucleic acid molecule of Claim 1 which encodes the amino acid sequence of SEQ ID NO:2.

28. An isolated nucleic acid molecule of Claim 1 having the sequence of SEQ ID NO:1.

29. An isolated nucleic acid molecule of Claim 1 encoding a (-)-limonene synthase.

30. An isolated nucleic acid molecule of Claim 1 encoding a gymnosperm (-)-limonene synthase.

31. An isolated nucleic acid molecule of Claim 1 encoding a Grand fir (-)-limonene synthase.

32. An isolated nucleic acid molecule of Claim 1 which encodes the amino acid sequence of SEQ ID NO:6.

33. An isolated nucleic acid molecule of Claim 1 having the sequence of SEQ ID NO:5.

39. An isolated (-)-camphene synthase protein.

40. An isolated gymnosperm (-)-camphene synthase protein of Claim 39.

41. An isolated Grand fir (-)-camphene synthase protein of Claim 39.

42. An isolated (-)-camphene synthase protein of Claim 39, said isolated (-)-camphene synthase protein comprising the amino acid sequence set forth in SEQ ID NO:65.

43. An isolated (-)- β -phellandrene synthase protein.

44. An isolated gymnosperm (-)- β -phellandrene synthase protein of Claim 43.

45. An isolated Grand fir (-)- β -phellandrene synthase protein of Claim 43.

46. An isolated (-)- β -phellandrene synthase protein of Claim 43, said isolated (-)- β -phellandrene synthase protein comprising the amino acid sequence set forth in SEQ ID NO:67.

47. An isolated terpinolene synthase protein.

48. An isolated gymnosperm terpinolene synthase protein of Claim 47.

49. An isolated Grand fir terpinolene synthase protein of Claim 47.

50. An isolated terpinolene synthase protein of Claim 47, said isolated terpinolene synthase protein comprising the amino acid sequence set forth in SEQ ID NO:78.

51. An isolated (-)-limonene/(-)- α -pinene synthase protein.

52. An isolated gymnosperm (-)-limonene/(-)- α -pinene synthase protein of Claim 51.

53. An isolated Grand fir (-)-limonene/(-)- α -pinene synthase protein of Claim 51.

54. An isolated (-)-limonene/(-)- α -pinene synthase protein of Claim 51, said isolated (-)-limonene/(-)- α -pinene synthase protein comprising the amino acid sequence set forth in SEQ ID NO:69.

55. An isolated myrcene synthase protein.

56. An isolated gymnosperm myrcene synthase protein of Claim 55.

57. An isolated Grand fir myrcene synthase protein of Claim 55.

58. An isolated myrcene synthase protein of Claim 55, said isolated myrcene synthase protein comprising the amino acid sequence set forth in SEQ ID NO:2.

59. An isolated (-)-limonene synthase protein.

60. An isolated gymnosperm (-)-limonene synthase protein of Claim 59.

61. An isolated Grand fir (-)-limonene synthase protein of Claim 59.

62. An isolated (-)-limonene synthase protein of Claim 59, said isolated (-)-limonene synthase protein comprising the amino acid sequence set forth in SEQ ID NO:6.

63. An isolated (-)-pinene synthase protein.

64. An isolated gymnosperm (-)-pinene synthase protein of Claim 63.

65. An isolated Grand fir (-)-pinene synthase protein of Claim 63.

66. An isolated (-)-pinene synthase protein of Claim 63, said isolated (-)-pinene synthase protein comprising the amino acid sequence set forth in SEQ ID NO:4.

67. (Amended) A replicable expression vector comprising a nucleic acid sequence encoding a monoterpene synthase selected from the group consisting of (-)-

camphene synthase, (-)- β -phellandrene synthase, terpinolene synthase, (-)-limonene/(-)- α -pinene synthase, myrcene synthase, and (-)-limonene synthase.

68. A replicable expression vector of Claim 67 comprising a nucleic acid sequence encoding a (-)-camphene synthase.

69. A replicable expression vector of Claim 67 comprising a nucleic acid sequence encoding a (-)- β -phellandrene synthase.

70. A replicable expression vector of Claim 67 comprising a nucleic acid sequence encoding a terpinolene synthase.

71. A replicable expression vector of Claim 67 comprising a nucleic acid sequence encoding a (-)-limonene/(-)- α -pinene synthase.

72. A replicable expression vector of Claim 67 comprising a nucleic acid sequence encoding a myrcene synthase.

73. A replicable expression vector of Claim 67 comprising a nucleic acid sequence encoding a (-)-limonene synthase.

75. A host cell comprising a vector of Claim 68.

76. A host cell comprising a vector of Claim 69.

77. A host cell comprising a vector of Claim 70.

78. A host cell comprising a vector of Claim 71.

79. A host cell comprising a vector of Claim 72.

80. A host cell comprising a vector of Claim 73.

82. A method of enhancing the production of a monoterpene synthase in a suitable host cell comprising introducing into the host cell an expression vector of Claim 67 under conditions enabling expression of the monoterpene synthase in the host cell.

83. The method of Claim 82 wherein said host cell is a plant cell.

84. The method of Claim 83 wherein said cell is from a plant selected from the group consisting of Brassica, cotton, soybean, safflower, sunflower, coconut, palm, wheat,

barley, rice, corn, oats, amaranth, pumpkin, squash, sesame, poppy, grape, mung beans, peanut, peas, beans, broad beans, chick peas, lentils, radish, alfalfa, cocoa, coffee, tree nuts, spinach, culinary herbs, berries, stone fruit and citrus.

85. The method of Claim 83 wherein said plant cell is a seed cell.

86. The method of Claim 83 wherein said plant cell is a leaf cell.

87. Seed comprising a cell produced by the method of Claim 85.

88. Seed of Claim 87 selected from the group consisting of seed of Brassica, cotton, soybean, safflower, sunflower, coconut, palm, wheat, barley, rice, corn, oats, amaranth, pumpkin, squash, sesame, poppy, grape, mung beans, peanut, peas, beans, broad beans, chick peas, lentils, radish, alfalfa, cocoa, coffee and tree nuts.

89. Oil extracted from the seed of Claim 88.

90. Meal extracted from the seed of Claim 88.

REMARKS

Please enter the foregoing claim amendments and amendments to the specification before examining the application.

Respectfully submitted,

CHRISTENSEN O'CONNOR
JOHNSON KINDNESS^{PLLC}



Barry F. McGurl
Registration No. 43,340
Direct Dial No. 206.695.1775

BFM:jlj

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS^{PLLC}
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

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Claims 34-38, 74, and 81 have been cancelled.

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CHRISTENSEN O'CONNOR JOHNSON KINDNESS^{PLLC}
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100